Reference: *Biol. Bull.* **186**: 202–212. (April, 1994)

Changes Occur in the Central Nervous System of the Nudibranch *Berghia verrucicornis* (Mollusca, Opisthobranchia) During Metamorphosis

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Abstract. The structure of the larval and juvenile central nervous system (CNS) in *Berghia verrucicornis*, an aeolid nudibranch, was examined using 1-μm serial sections. The CNS consists of paired optic, cerebral, pleural (also known as sub- and supra-intestinal ganglia), pedal, and buccal ganglia, and a single visceral ganglion. A pleurovisceral loop is present. The organization of the CNS changes as the nudibranch undergoes metamorphosis. In general, there is a condensation of the CNS. The cerebral and pleural ganglia fuse to form the prominent cerebropleural ganglia. The single visceral ganglion fuses with the pleural portion of the left cerebropleural ganglion. The buccal ganglia enlarge and fully organize into a cortex of nerve cell bodies and medulla of nerve fibers. Rhinophoral ganglia develop anterior to each cerebropleural ganglion and a pair of nervous processes extend from each: one to the developing rhinophore and the other anteroventral toward the mouth and associated structures. These metamorphic changes are similar to those seen in other commonly studied opisthobranch species, suggesting that *Berghia verrucicornis* is an appropriate model for the developmental examination of structure and function in molluscan nervous systems.

Introduction

The use of opisthobranch mollusks for the investigation of neurobiological questions as diverse as the differentiation of neurons (Schacher *et al.*, 1979; McAllister *et al.*, 1983; Schacher, 1983; Bulloch, 1985), the connections between specific, individual neurons (Kandel *et al.*, 1967; Schacher, 1983), and the function of neurotransmitters during neurodevelopment (Goldberg and Kater, 1989) has contributed to our knowledge of the organization of neuronal systems. Opisthobranchs possess several unique features that make them useful as neural models. For instance, the relative simplicity of the nervous system and the accessibility of their neuron perikarya make opisthobranchs exceedingly convenient models for correlating animal behavior with nervous activity, as well as for delving into the cellular and molecular mechanisms driving the development and function of the nervous system (see Willows, 1971, 1973; Nagle *et al.*, 1989a, b; Baux *et al.*, 1990; Bedian *et al.*, 1991; Hickmott and Carew, 1991; Ziv *et al.*, 1991). Also, the fact that some aspects of the organogenesis of the molluscan central nervous system (CNS) resemble those of the vertebrate peripheral nervous system (PNS) may enable investigators to draw parallels between the ontogeny of the easily studied opisthobranch CNS and the more complex vertebrate PNS (Jacob, 1984; Bulloch, 1985; Cash and Carew, 1989).

In the past, to fully exploit opisthobranch mollusks as neurobiological models, it was necessary to devise protocols for maintaining the nudibranchs in the laboratory throughout their life cycle or to obtain the appropriate stage animal from the field as needed. A number of attempts at culturing these organisms in the laboratory have been successful (Kriegstein *et al.*, 1974; Bridges, 1975; Harris, 1975; Kempf and Willows, 1977; Switzer-Dunlap and Hadfield, 1977; Bickell [=Page] and Kempf, 1983; Paige, 1988); however, such culture work has been essentially limited to marine laboratories

Received 13 September 1992; accepted 24 January 1994.

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Abbreviations: CNS = central nervous system; MFSA = Millipore-filtered (0.45 μm), seasoned, aquarium water; PNS = peripheral nervous system.

where fresh seawater and prey organisms are readily available.

Berghia verrucicornis is suitable for culture in inland laboratories because it has lecithotrophic larvae (the larvae derive energy from endogenous yolk reserves), it does not require a specific metamorphic inducer, and it feeds on the coelenterate *Aiptasia pallida,* a species amenable to laboratory culture (Hessinger and Hessinger, 1981). Thus, we are able to rear and maintain this nudibranch through successive generations at our inland laboratory by using artificial seawater and relatively simple techniques (Carroll and Kempf, 1990).

In this paper, as a prelude to the use of this species in neurodevelopmental studies, we describe the neuromorphology of two stages in the life history of *B. verrucicornis:* the veliger larval stage and the prefeeding juvenile stage. These stages occur approximately 12 and 14 days after oviposition at 22°C. The larval stage was chosen for examination because it represents the most developed stage in the CNS before metamorphosis. The prefeeding juvenile stage was selected to assess changes that occur in the gross neuromorphology of the CNS during and just after metamorphosis.

Materials and Methods

Animal culture

Animals were cultured according to the methods of Carroll and Kempf (1990). Briefly, adult nudibranch pairs were kept in bowls of Millipore-filtered (0.45 μm), seasoned aquarium water (MFSA) and fed the sea anemone *Aiptasia pallida* as needed. The culture bowl and MFSA were changed daily. Newly layed egg masses were removed to aerated egg mass cultures containing 300 ml of MFSA. Upon hatching from the egg mass, larvae were transferred to a metamorphosis culture dish containing several tiny *A. pallida.* Individual larvae began to metamorphose as soon as 1 day after hatching, and most of the larvae had reached the juvenile stage by 5 days after hatching. Juveniles began feeding soon after metamorphosis (3–5 days) and reached adulthood (indicated by the laying of their first egg mass) approximately 60 days after oviposition.

For culture or histological examination, veliger larvae were collected either by mechanically disrupting the egg mass at 11 days after oviposition or by concentrating larvae that had already hatched. One-day postmetamorphic individuals were gathered directly from the metamorphosis cultures. These two stages were then treated as described below.

Fixation and histological examination of pre- and postmetamorphic stages

Larvae and juveniles were relaxed in a 1:3 mixture of seawater and a saturated solution of chlorobutanol in MFSA. The specimens were fixed for 1 h in a primary fixative of 2.5% glutaraldehyde in 0.2 *M* Millonig's phosphate buffer and 0.14 *M* NaCl. Larval shells were decalcified by one of two methods: (1) before fixation, live larvae were treated with 2-[N-morpholino] ethanesulfonic acid (MES) in MBL artificial seawater according to the methods of Pennington and Hadfield (1989), or (2) after primary fixation, the larvae were placed into a 1:1 solution of 10% sodium-ethylenediaminotetraacetic acid (Na-EDTA) and primary fixative. Following primary fixation and decalcification, the specimens were rinsed in buffer and then secondarily fixed in a solution of 2% osmium tetroxide in 1.25% sodium carbonate buffer. After secondary fixation, specimens were washed in 1.25% sodium carbonate buffer and then dehydrated through an ethanol series to 100% propylene oxide before being infiltrated with a Poly/Bed 812-propylene oxide mixture and embedded in pure Poly/Bed 812 (Polysciences). The specimens were embedded in flat dishes by spreading them in a thin layer of plastic and curing overnight at 60°C. They were then cut out of the plastic mold and glued to metal studs for sectioning.

Serial sections of 1 μm were cut on a Reichart ultramicrotome, mounted on gelatin-coated glass slides, and stained with methylene blue-Azure II (Richardson *et al.,* 1960) or 1% Thionin in distilled water.

Ganglia and connective designations

Precise ganglia designations have proven extremely difficult in light of recent investigations by Page (1992a, b). Historically, the opisthobranch larval CNS has been described as a circumenteric ring consisting of the paired cerebral, pedal, and pleural ganglia linked by connectives around the esophagus (see Dorsett, 1986). A pair of buccal ganglia also exist in this region and are connected to the cerebral ganglia via the cerebrobuccal connectives. Extending from the pleural ganglia is a pleurovisceral loop that runs posterolateral, extending to the supraintestinal (right) and subintestinal (left) ganglia, and to the single visceral ganglion. In some opisthobranch larvae, the parietal ganglia are between the pleural and intestinal ganglia; in a few opisthobranchs, another ganglion, the osphradial, has been described as connected to the supraintestinal ganglion but outside of the pleurovisceral loop (Kriegstein, 1977; Page, 1992a). Metamorphosis entails an alteration in the CNS of opisthobranchs. In the anaspid *Aplysia californica,* the connectives lengthen and spread the ganglia further apart (Kriegstein, 1977). The opposite has been true for nudibranchs in which the cerebral and pleural ganglia fuse to form the large cerebropleural ganglia and the pleurovisceral loop shortens (Thompson, 1958, 1962; Tardy, 1970, 1974; Bonar and Hadfield, 1974; Kriegstein, 1977; Bonar, 1978). The above "blueprint"

of the opisthobranch CNS has arisen from histological studies on a variety of species.

Recently, Page (1992a, b) suggested a reinterpretation of the opisthobranch nervous system based upon an ultrastructural study of several stages in the life cycle of the nudibranch *Melibe leonina*. Ganglia were identified by comparing the placodal origin of their constituent neurons to those described for ganglia of developing prosobranchs. Using this approach, Page described a CNS that differs considerably from the generally accepted nudibranch plan. The cerebral ganglia of larval *M. leonina* are linked to the fused pleuropedal ganglia in the larval foot via two connectives: the cerebropedal and the cerebropleural. The visceral loop arises directly from the cerebral ganglia and consists of the supraintestinal ganglion (on the right, this may actually contain the parietal ganglion also), the osphradial ganglion (which lies outside the visceral loop but is connected to the supraintestinal ganglion), the subintestinal ganglion (on the left), and the single visceral ganglion. The novel notion in this interpretation is that the pleural ganglion lies outside of the visceral loop, and it fuses with the pedal ganglion in the larva. Metamorphosis in *M. leonina* also entails fusion of the constituent ganglia and incorporation of the visceral loop components into the cerebral ganglia. In this interpretation (Page, 1992a, b), the subintestinal and visceral ganglia fuse with the left cerebral ganglion and the supraintestinal ganglion fuses with the right cerebral ganglion, creating the left and right cerebroabdominal ganglia.

The data presented in the two Page studies (1992a, b) support her conclusions fully; however, the idea that ganglion designations based upon comparison to ectodermal ingression sites for developing prosobranch ganglia apply to all opisthobranchs is premature. Indeed, the fact that the pleural ganglion lies within the visceral loop of the CNS in some prosobranch adults lends some uncertainty to the global applicability of this scheme (Graham, 1985).

For the purposes of this study, we have chosen to remain with the classical designations for the components of the *B. verrucicornis* CNS for three reasons. First, the nomenclature used in the classical scheme is based upon studies of many opisthobranch species, and while it is true that the descriptions differ in some details, the basic layout provides ganglion identifications that will be familiar to most investigators. Second, the new interpretation by Page (1992a, b) introduces a novel concept (*i.e.*, the pleural ganglia are separate from the visceral loop) that, although supported by seemingly convincing evidence, has not yet become the accepted model for the opisthobranch CNS. Third, our primary purpose in this paper is to introduce *B. verrucicornis* as an appropriate model for future neurodevelopmental studies.

Measurements

Three measurements were made to determine whether the CNS of *B. verrucicornis* condensed during metamorphosis. These measurements were (1) the distance from the most anterior point of the organism to the most posterior aspect of the pleural ganglion divided by the total length of the same organism (Pd); (2) the length of the cerebropleural connective along the anterior-posterior axis; and (3) the distance from the most anterior aspect of the cerebral ganglion to the most posterior aspect of the pleural ganglion (AC-PP). The ganglia measured were those on the left side in both larvae and juveniles. A Student's *t*-test was used to determine any difference between the means at the 95% confidence level.

Results

Life history of Berghia verrucicornis

B. verrucicornis adults lay their egg masses as spirals containing several hundred embryos. The embryos develop and obtain the appearance of competence (*e.g.*, eyespots, retracted mantle, propodium) within the egg mass. Hatching occurs 11–12 days after oviposition, and the lecithotrophic larvae spend 1–5 days as swimming veligers. Larvae of *B. verrucicornis* are lecithotrophic and are competent to metamorphose as early as 1 day after they hatch. As the larvae approach metamorphosis, they generally settle to the bottom of the culture dish and attach to the substratum. Metamorphosis entails, in gross morphological terms, the loss of the larval shell and velum, as well as a reorganization of the general body plan (Fig. 1). Adulthood is attained approximately 60 days after oviposition. For a more complete description of the life history of this species, see Carroll and Kempf (1990).

Larval nervous system

At hatching, the larvae of *B. verrucicornis* possess all of the major ganglia commonly described (see *Ganglia and connective designations*, Materials and Methods) in competent nudibranch larvae (Thompson, 1958, 1962; Bickell [=Page] and Chia, 1979; Bickell [=Page] and Kempf, 1983; Kempf *et al.*, 1987; Page, 1992a, b). These ganglia are paired, with the exception of the single visceral ganglion, and have a cortex of nerve cell bodies and a medulla of nerve fibers (neuropil). The largest at hatching are the cerebral ganglia, located dorsal to the statocysts (Fig. 2a). Nerve fibers pass medially out of each cerebral ganglion to become part of the cerebral commissure that connects the left and right cerebral ganglia dorsal to the esophagus (Fig. 2b).

The cerebral ganglia are joined, via connectives, to at least three other pairs of ganglia: the optic, pedal, and pleural. Anterodorsal to the cerebral ganglia are the de-

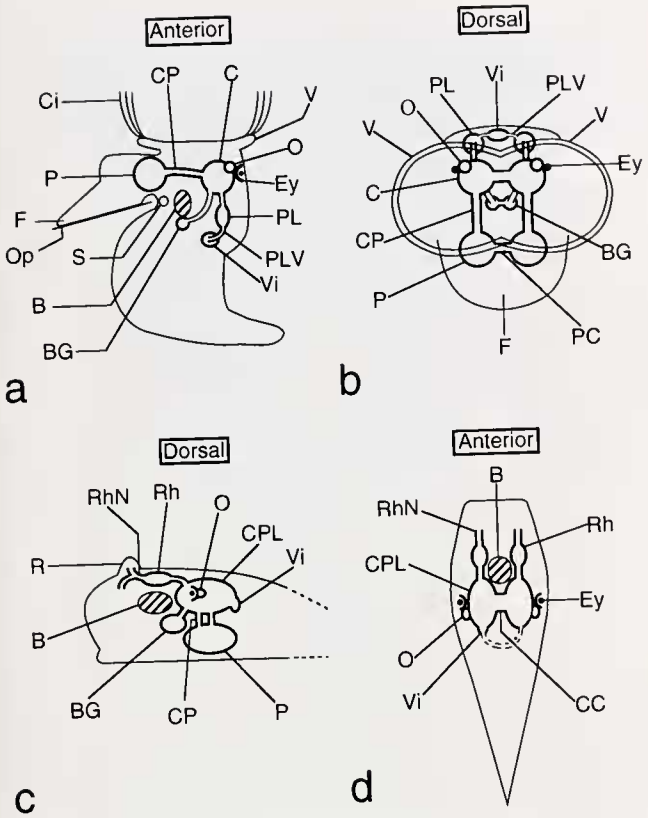


Figure 1. Schematic diagram of the veliger larva and prefeeding juvenile of *Berghia verrucicornis* showing the spatial relationship of the CNS to various larval and juvenile structures. (a) Sagittal view of the larva's left side. In the larva, the cerebral and pleural ganglia are discrete structures that are linked by the cerebropleural connective. The visceral ganglion lies near the midline of the larva and is connected to the left pleural ganglion by the pleurovisceral loop. The shell, which covers the ventral surface of the larva posterior to the foot and the entire dorsal surface, has been omitted in the drawing. The buccal ganglia lie posterior to the buccal mass. (b) Transverse view of the anterior end of the larva shown in (a). The buccal, cerebral, optic, pedal, and pleural ganglia are viewed through the velum. The visceral ganglion is depicted dorsal to the velum in this drawing. The portion of the pleurovisceral loop connecting the right pleural ganglion to the visceral ganglion is assumed to be present, but was not clearly discernible in 1-μm sections. (c) Sagittal view of the left side of a prefeeding juvenile. In the juvenile, the cerebral and pleural ganglia are fused to form the cerebropleural ganglia. As indicated, the visceral ganglion is tightly associated with the left cerebropleural ganglion at this stage and later will be completely incorporated into the left cerebropleural ganglion. The cerebropleural ganglia have now receded posterior to the buccal mass and new ganglia, the rhinophoral ganglia, have appeared between the cerebropleural ganglia and the anterior epidermis. Each buccal ganglion is now notably larger than it appeared in the larva. The dotted lines at the posterior end of the juvenile represent its body as it tapers towards the tail. (d) Frontal view from the dorsal surface of the juvenile. As noted above, the visceral ganglion has become tightly associated with the left cerebropleural ganglion. The pleurovisceral loop is assumed to extend to the right cerebropleural ganglion, but could not be traced along its entire length in 1-μm sections. B = buccal mass; BG = buccal ganglion; C = cerebral ganglion; CC = cerebral commissure; Ci = velar cilia; CP = cerebropedal connective; CPL = cerebropleural ganglion; Ey = eyespot; F = foot; O = optic ganglion; Op = operculum; P = pedal ganglion; PC = pedal commissure; PL = pleural ganglion; PLV = portion of the pleurovisceral loop; R = rhinophore; Rh = rhinophoral ganglion; RhN = rhinorphoral nerve; S = statocyst; V = velum; Vi = visceral ganglion.

veloping optic ganglia. Located lateral, and a little posterior, to each optic ganglion, are eyespots consisting of a pigmented retina and spherical lens. Ventral to the cerebral ganglia, the cerebropedal connectives pass anterior to the statocysts and join the pedal ganglia in the most dorsal portion of the larval foot (Fig. 2a). The pedal ganglia, consisting of a well-developed cortex and neuropil, are directly ventral to the statocysts and are connected to each other via the pedal commissure.

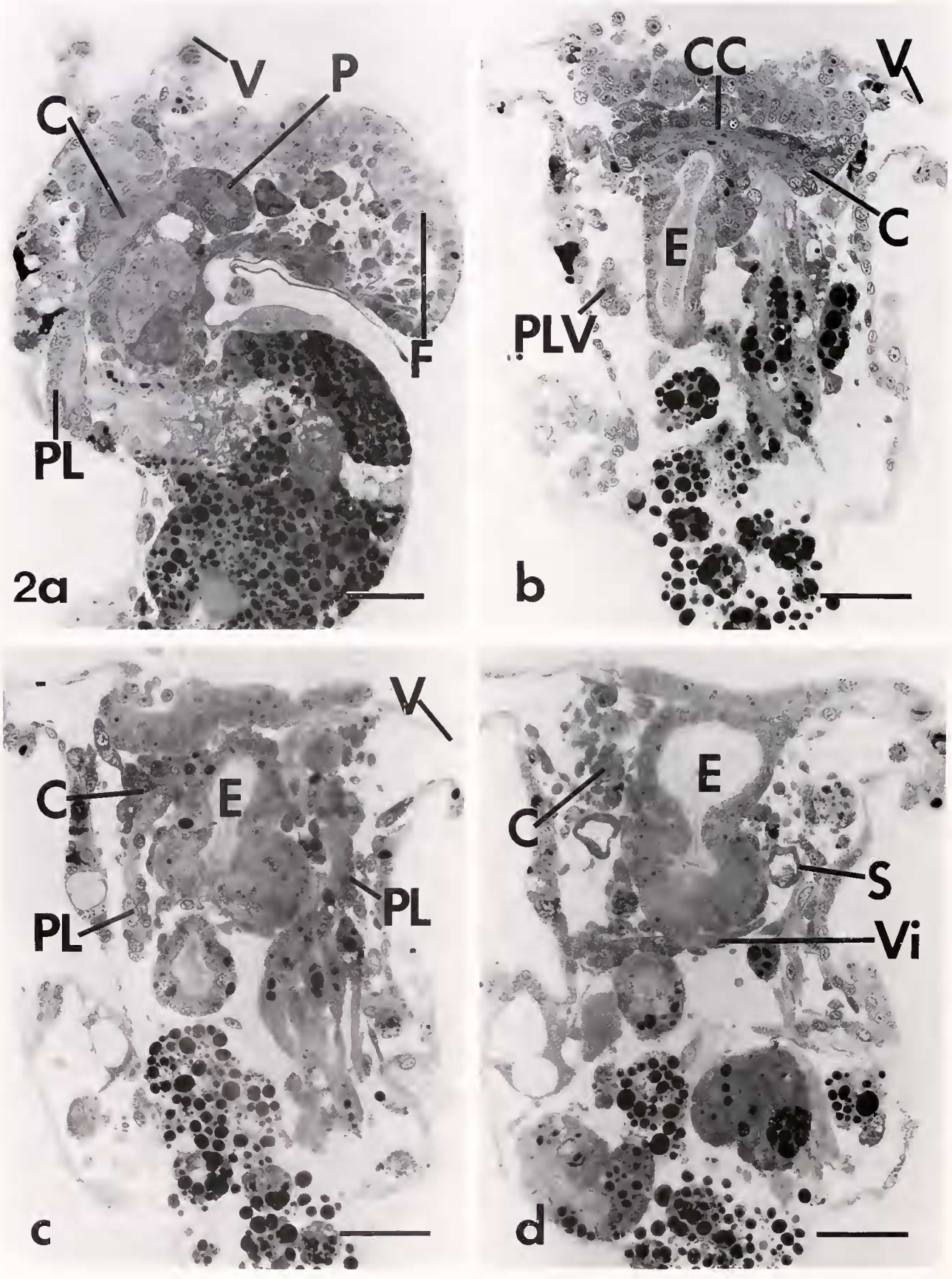
Immediately posterior to their respective left and right cerebral ganglia are the left and right pleural ganglia (Fig. 2c). Nerve fibers from the cerebral neuropils pass posterior through a short cerebropleural connective to their respective pleural ganglia. A connective between the pleural and pedal ganglia in the larva was not clearly discernible in our sections. Continuing posterior from the left and right pleural ganglia are connectives that run posteromedially to the single visceral ganglion (Fig. 2d) to complete the pleurovisceral loop. The visceral ganglion is located along the midline of the larva, immediately anterior to the visceral mass.

The fifth pair of ganglia in *B. verrucicornis* larvae are the buccal ganglia. These are located to the left and right of the developing buccal mass and medioventral to the pleural ganglia. Presumably, these ganglia are linked to the cerebral ganglia, although we have not positively identified these connectives in the larva. The buccal commissure connects the left and right buccal ganglia posterior to the buccal mass.

The postmetamorphic central nervous system

Changes in the postmetamorphic nervous system are most evident in the fusion of the ganglia and the corresponding condensation of the CNS (see below). The cerebral and pleural ganglia are fused into a single, large cerebropleural ganglion on both the left and right sides of the juvenile (Fig. 3a, b). The left and right cerebropleural ganglia are joined to their respective pedal ganglia via two connectives. Each connective appears to join a *distinct* neuropil in the cerebropleural ganglia; presumably these are the neuropils of the cerebral and pleural ganglia that are still undergoing the process of fusion. We consider the anterior connective on each side to be the cerebropedal connective and the posterior connective to be the pleuropedal connective. All the paired ganglia become more spherical than they are in the larval stage. The relative position of the optic ganglia, with respect to the eyespots and the cerebropleural ganglia, changes during metamorphosis. These ganglia are located anterolateral to the cerebral ganglia and immediately posterior to the eyespots in the postmetamorphic central nervous system.

New structures appear in the postmetamorphic nervous system. As mentioned above, the pleuropedal connectives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are now visible just anterior to the statocysts linking the pleural portion of the cerebropleural ganglia to the pedal ganglia. The cerebrobuccal connectives are now apparent, passing ventrally from each cerebropleural ganglion to its respective buccal ganglion. The buccal ganglia are located anteromedial to the statocysts, ventromedial to the cerebropleural ganglia, and dorsomedial to the pedal ganglia (Fig. 3c). Connectives join a new pair of ganglia, the rhinophoral ganglia, to their respective cerebropleural ganglia (3a, d). These rhinophoral ganglia are anterior to the cerebropleural ganglia, just beneath the dorsal epidermis (Fig. 3d) and are first seen in the newly metamorphosed juvenile. A nerve can be seen exiting each rhinophoral ganglion and extending into the developing rhinophores. Also arising from each rhinophoral ganglion is a nervous process that proceeds anteroventral, perhaps to the mouth, salivary glands, or oral tentacles. The visceral ganglion is now tightly associated with the left cerebropleural ganglion. The pleurovisceral loop can be followed from the visceral ganglion towards the right side of the juvenile; however, its connection to the right cerebropleural ganglion is not clearly evident. The pedal ganglia, located in the anterior region of the juvenile foot, have definite nervous processes passing into anterior and posterior portions of the foot.

Evidence for the condensation of the CNS during metamorphosis

Some of the morphological data presented above suggest that the CNS of *B. verrucicornis* condenses during metamorphosis. Evidence of condensation is seen in the fusion of the cerebral ganglia to their respective pleural ganglia in the 1-day postmetamorphic juvenile and the tight association between the visceral ganglion and the left cerebropleural ganglion in the juvenile (Fig. 4).

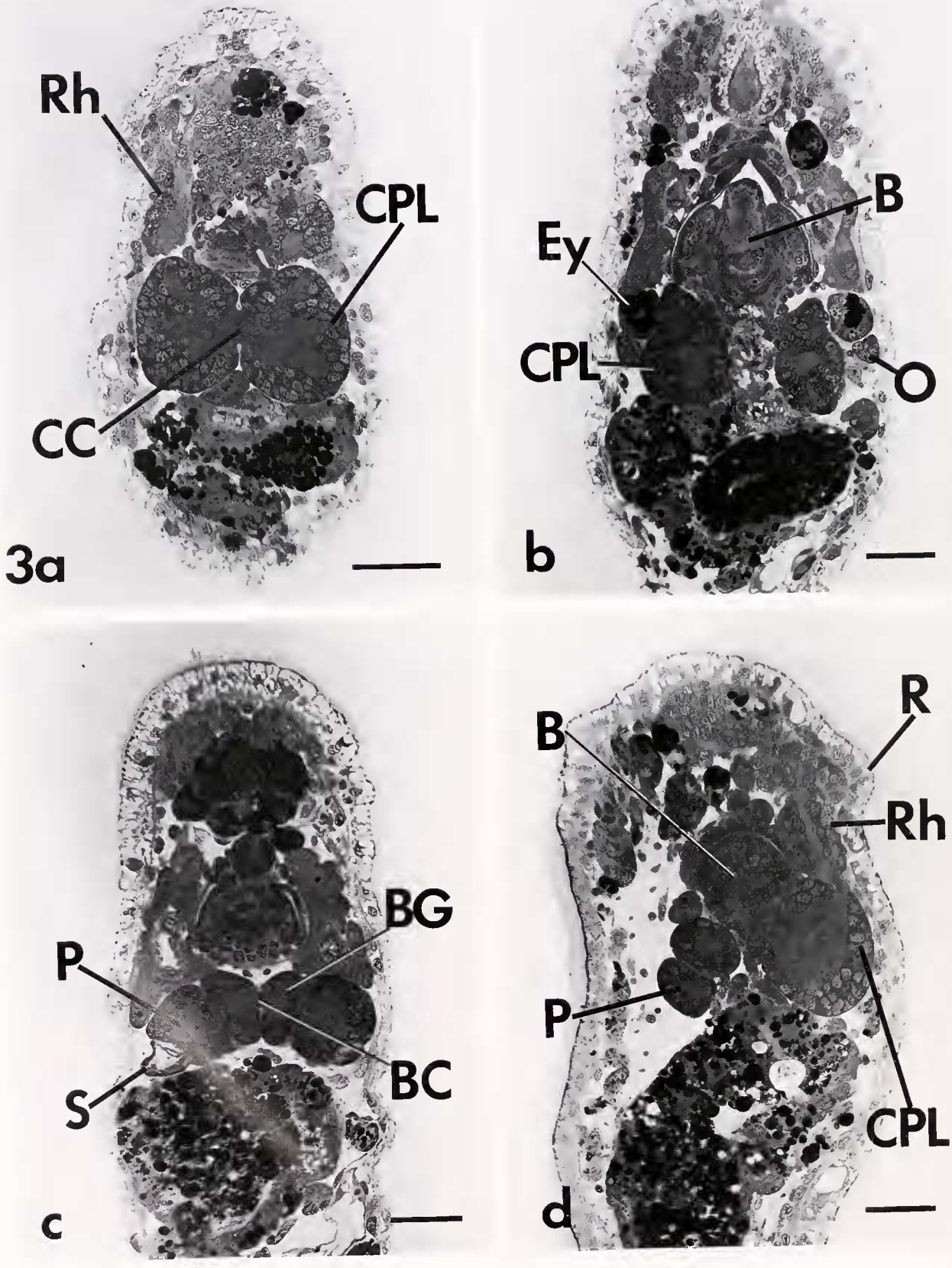
Three measurements were made to determine if the central nervous system had become more compact during metamorphosis (Table I). The first, Pd, determined the relative position of the cerebral and pleural ganglia in the larva and juvenile by dividing the distance from the most anterior aspect of the organism (larva and juvenile) to the posterior aspect of the pleural ganglion by the total length of the organism. (If the pleural ganglion was located in the most posterior portion of the organism, this value would be equal to 1. The more anterior the CNS is located, the smaller the number.) The relative position of these ganglia was the same in larva (0.51 ± 0.05, mean $\pm$ S.D., $n = 4$) and juvenile (0.45 ± 0.08). The second measurement recorded the length of the cerebropleural connective along the anterior-posterior axis. In this case, condensation of the CNS is evident even without measurement because the length of these connectives is reduced from 13.5 ± 5.1 μm in the larva to nothing in the juvenile by the fusion of the cerebral and pleural ganglia during metamorphosis. Finally, determination of the distance from the most anterior aspect of the cerebral ganglia to the most posterior aspect of the pleural ganglia (AC-PP) demonstrated that this distance was significantly greater in the larva (74.1 ± 13 μm) than in the juvenile (54.5 ± 5.0 μm).

Discussion

As discussed above, the nomenclature used to identify ganglia in opisthobranchs is in a state of flux, mainly due to the recent investigations of Page (1992a, b). In addition, other past studies are not in full agreement on ganglion identification and larval CNS structure (*e.g.*, Tardy, 1970, 1974). To assist in relating our interpretation of the structure of the larval and juvenile CNS in *Berghia verrucicornis* to that of past investigators' descriptions for other species of opisthobranchs, these various larval CNS morphologies are summarized in Figure 5.

In *B. verrucicornis*, the structures that constitute most of the juvenile and adult CNS are present when the larvae are released from the egg mass. These are the cerebral, optic, pedal, pleural, buccal, and visceral ganglia. The first five of these ganglia are paired, whereas the sixth, the visceral ganglion, is single (see below). The dendronotid *Tritonia diomedea*, which has a planktotrophic larval stage, exhibits a similar complement of ganglia as its larva approaches metamorphic competence; however, only the cerebral ganglia are present at hatching (Kempf *et al.*, 1987). The other ganglia develop during the larval life of this species. Another opisthobranch with a planktotrophic

Figure 2. Sections (1 μm) of a competent *Berghia verrucicornis* larva (the anterior end of the organism is toward the top of the page). (a). Sagittal section showing the relationship between the pedal, cerebral, and pleural ganglia. Notice the distance between the cerebral and pleural ganglia. These ganglia are distinct in the larval stage but fuse to form a single cerebropleural ganglion during metamorphosis. (b). Frontal section illustrating the cerebral commissure and its location adjacent to the anterior epidermis. (c). Frontal section depicting the connection between the right cerebral ganglion and the right pleural ganglion. (d). Frontal section showing the relationship of the visceral ganglion to the esophagus. This ganglion is part of the pleurovisceral loop. C = cerebral ganglion; CC = cerebral commissure; E = esophagus; F = larval foot; P = pedal ganglion; PL = pleural ganglion; PLV = portion of the pleurovisceral loop; S = statocyst; V = velum; Vi = visceral ganglion. Scale bar = 35 μm.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larva, the nudibranch *Melibe leonina*, has cerebral ganglia linked to primordia of the pedal ganglia via cerebropedal connectives at hatching (Page, 1992a, b). Also present at this stage is a complete pleurovisceral loop, though other ganglia commonly associated with this structure are absent at this early stage (Page, 1992a, b). As with *T. diomedea*, the other ganglia develop during the larval period. A similar scenario of CNS development is seen in the anaspid *Aplysia californica* (Kriegstein, 1977). The contrast in the timing of ganglion development between these species and *B. verrucicornis* reflects differences between the two developmental strategies (planktotrophic *vs.* lecithotrophic), rather than any real difference in the development of the central nervous system.

Some real differences are seen between the larval CNS in *A. californica* and *B. verrucicornis*. Kriegstein (1977) noted a pair of abdominal ganglia in *A. californica* that existed as part of a pleurovisceral loop and were located immediately anterior to the viscera. The right abdominal ganglion is described as a homolog of the supraintestinal ganglion, and the left as a combination of the subintestinal and visceral ganglia (Kriegstein, 1977). The visceral ganglion of *B. verrucicornis* may represent the fusion of one or more of these ganglia. Also present in *A. californica*, but not observed in *B. verrucicornis*, is the osphradial ganglion that is linked dorsally to the right abdominal ganglion by a short connective (Kriegstein, 1977).

The presence of a single visceral ganglion is not unique to *B. verrucicornis*. In the nudibranch *T. diomedea*, Kempf *et al.* (1987) note a single visceral ganglion that constitutes a part of the pleurovisceral loop. In *Aeolidiella alderi*, Tardy (1970, 1974) recognizes a single abdominal ganglion that occurs in a well-developed pleurovisceral loop. The relative location of the abdominal ganglion in this species and its association with other ganglia suggest that it is homologous to the visceral ganglion in *B. verrucicornis*. Though Bickell (=Page) and Kempf (1983) did not report a visceral ganglion in competent larvae of *M. leonina*, more recent examination of this species' larva by Page has established its presence (1992a).

In this study we are treating the pleural ganglion as part of the visceral loop. The designation for this ganglion is the most difficult to justify, because a clear pleuropedal connective was not identified in the veliger larva; however, examination of the postmetamorphic CNS reveals a distinct connective between what we have designated the pleural (posterior) portion of the cerebropleural ganglia and their respective pedal ganglia. It is possible that all connections between major ganglia are not fully developed in larvae of *B. verrucicornis*, because we also failed to find definitive cerebrobuccal connectives in the competent veliger; however, these connectives were well developed in the one-day postmetamorphic juvenile. A similar situation may exist in the development of the *A. californica* CNS. Kriegstein (1977, p. 375) describes the pleuropedal connective as merging with the cerebropedal connective before passing into the pedal ganglion in the late larval stage of *A. californica*, but shows a distinct pleuropedal connective in the corresponding drawing (Kriegstein, 1977, Fig. 1a). In both cases, however, the pleural ganglia are included in the pleurovisceral loop.

An alternative explanation is that we do not resolve a pleuropedal connective in the larva because it is not present, and what we have designated the pleural ganglia are, as suggested by Page (1992a, b), the supra- and subintestinal ganglia. Furthermore, the appearance of another connective between the cerebral and pedal ganglia in the juvenile is actually the cerebropleural connective arising from the pleural ganglion, which has fused with the pedal ganglion. This interpretation is supported by the recent Page model for *M. leonina* (Page, 1992a, b); however, we are not convinced this is the case in *B. verrucicornis*. The connectives extending to the pedal ganglion on both the left and right sides appear to emerge from distinct neuropils in what we have designated the fused cerebropleural ganglion, while each pedal ganglion clearly possesses a single neuropil. This is suggestive of a fusion event between the former cerebral and pleural ganglia.

Tardy (1970, 1974) uses different terminology to describe the ganglia of the pleurovisceral loop in *Aeolidiella alderi;* some of these ganglia are clearly identical to those in *B. verrucicornis*. Contrary to many published descriptions (Kriegstein, 1977; Bickell [=Page] and Kempf, 1983; Kempf *et al.*, 1987; Page 1992a, b), Tardy (1970, 1974) considered the cerebral and pleural ganglia to be a single fused ganglion on the left and right sides of the competent

Figure 3. Sections (1 μm) of a 1-day postmetamorphic *Berghia verrucicornis* juvenile (the anterior end of the organism is towards the top of the page). (a) Frontal section showing the cerebral commissure. The cerebral commissure is located more posteriorly in the juvenile than in the larva, and the cerebral and pleural ganglia have fused to form the cerebropleural ganglia. (b). Frontal section through the cerebral and optic ganglia. The optic ganglia are positioned lateral to the cerebropleural ganglia and posterior to the eyespots. (c). Frontal section at the level of the statocysts. (d). Sagittal section displaying the newly formed right rhinophoral ganglion. The rhinophore, a sensory organ in the adult, is beginning to form anterodorsal to the rhinophoral ganglion. B = buccal mass; BC = buccal commissure; BG = buccal ganglion; CC = cerebral commissure; CPL = cerebropleural ganglion; Ey = eyespot; O = optic ganglion; P = pedal ganglion; R = rhinophore; Rh = rhinophoral ganglion; S = statocyst. Scale bar = 25 μm.

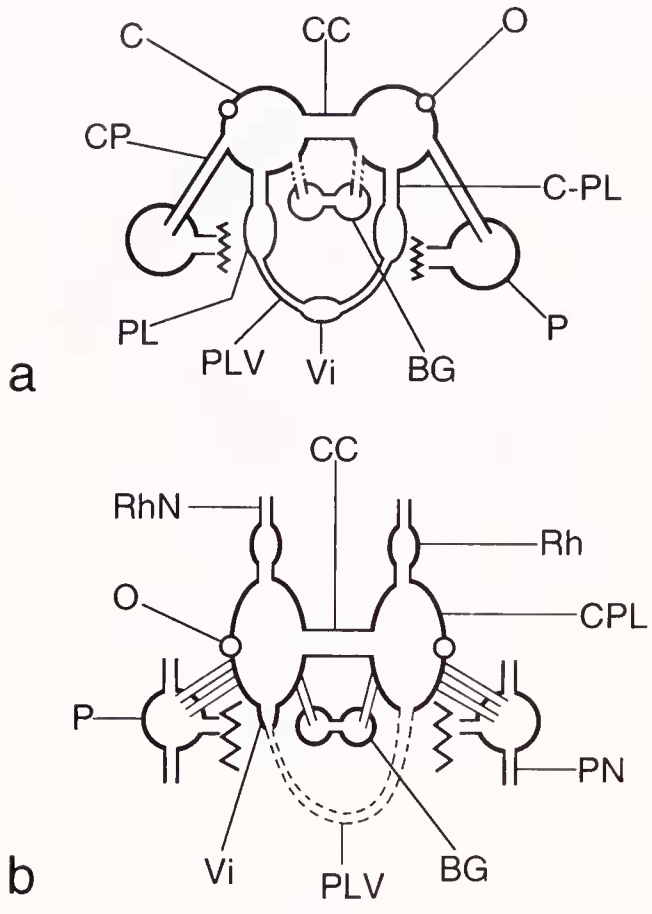

Figure 4. Diagram illustrating the organization of the (a) competent larval (11–12 days after oviposition) and (b) 1-day postmetamorphic (13–15 days after oviposition) CNS of *Berghia verrucicornis*. The anterior portion of the CNS is positioned toward the top of the page. In the 1-day postmetamorphic juvenile the cerebral and pleural ganglia are fused, and the visceral ganglion is being incorporated into the left cerebropleural ganglion. The dotted lines indicate portions of the cerebrobuccal connectives (a) and the pleurovisceral loop (b) that are assumed to be present, but could not be traced in 1-μm sections. The pedal commissure has been separated at the points indicated by the jagged lines. BG = right buccal ganglion; C = left cerebral ganglion; CC = cerebral commissure; CP = cerebropedal connective; CPL = right cerebropleural ganglion; C-PL = right cerebropleural connective; O = optic ganglion; P = pedal ganglion; PL = left pleural ganglion; PLV = portion of the pleurovisceral loop; PN = pedal nerve; Rh = rhinophoral ganglion; RhN = rhinophoral nerve; Vi = visceral ganglion.

veliger of *Aeolidiella alderi*. Just posterior to these putative cerebral ganglia, that author identified the left and right parietal ganglia. These ganglia were connected by a parietalvisceral loop that, moving from left to right, included infraintestinal, abdominal, and supraintestinal ganglia. The relative location of the pleural ganglia we have described in *B. verrucicornis* is the same as the parietal ganglia described by Tardy (1970, 1974) in larvae of *Aeolidiella alderi*. Presumably, in *B. verrucicornis*, the abdominal and the supra- and infraintestinal ganglion described by Tardy (1970, 1974) in *Aeolidiella alderi* are either fused with the visceral ganglion or with the pleural ganglia.

Other studies have reviewed the changes that occur in the opisthobranch central nervous system during metamorphosis (Marois and Carew, 1990). Many of these events are similar to what we observed in *B. verrucicornis*. In summary, (1) the central ganglia become more concentrated during metamorphosis (Thompson, 1958, 1962; Tardy, 1970, 1974; Bonar and Hadfield, 1974; Bonar, 1978; Page, 1992a, b). (2) The cerebral ganglia migrate from their position anterior to the buccal mass to lie above the buccal mass in the juvenile (Thompson, 1958, 1962; Tardy, 1970, 1974; Kriegstein, 1977). (3) The components of the pleurovisceral loop, namely the left pleuro-visceral connective and the visceral ganglion, are incorporated into the pleural portion of the left cerebropleural ganglion. (4) Similar to descriptions for *M. leonina*, new neural structures, the rhinophoral ganglia and associated connectives and nerves, are apparent in the newly metamorphosed juvenile of *B. verrucicornis* (Bickell [=Page] and Kempf, 1983; and Page, 1992a, b).

The short embryonic, larval, and juvenile life of *Berghia verrucicornis* and the ability to rear this nudibranch in the laboratory will make it a convenient model for neurodevelopmental studies. We are currently investigating the expression of specific neurotransmitters during development of *B. verrucicornis*, with the eventual aim of correlating neurotransmitter distribution and neuroanatomy with function.

Table 1

Measurements of the larval and juvenile CNS

	Pd[a]	Cerebropleural connective (μm)[b]	AC-PP (μm)[b,c]
Larval I.D. #			
390#2	0.48	12.2	68.6
590#11	0.49	7.4	63.7
590#12	0.49	19.6	71.1
590#14	0.59	14.7	93.1
Juvenile I.D. #			
590#4	0.46	0	58.8
590#5	0.39	0	58.8
590#6	0.40	0	51.4
590#7	0.56	0	49.0

[a] Pd = the distance from the most anterior aspect of the organism to the most posterior aspect of the pleural ganglion divided by the total length of the same organism.

[b] There is a significant difference between the larva and juvenile for this measurement ($P < 0.05$).

[c] AC-PP = the distance from the most anterior aspect of the cerebral ganglion to the most posterior aspect of the ipsilateral pleural ganglion.

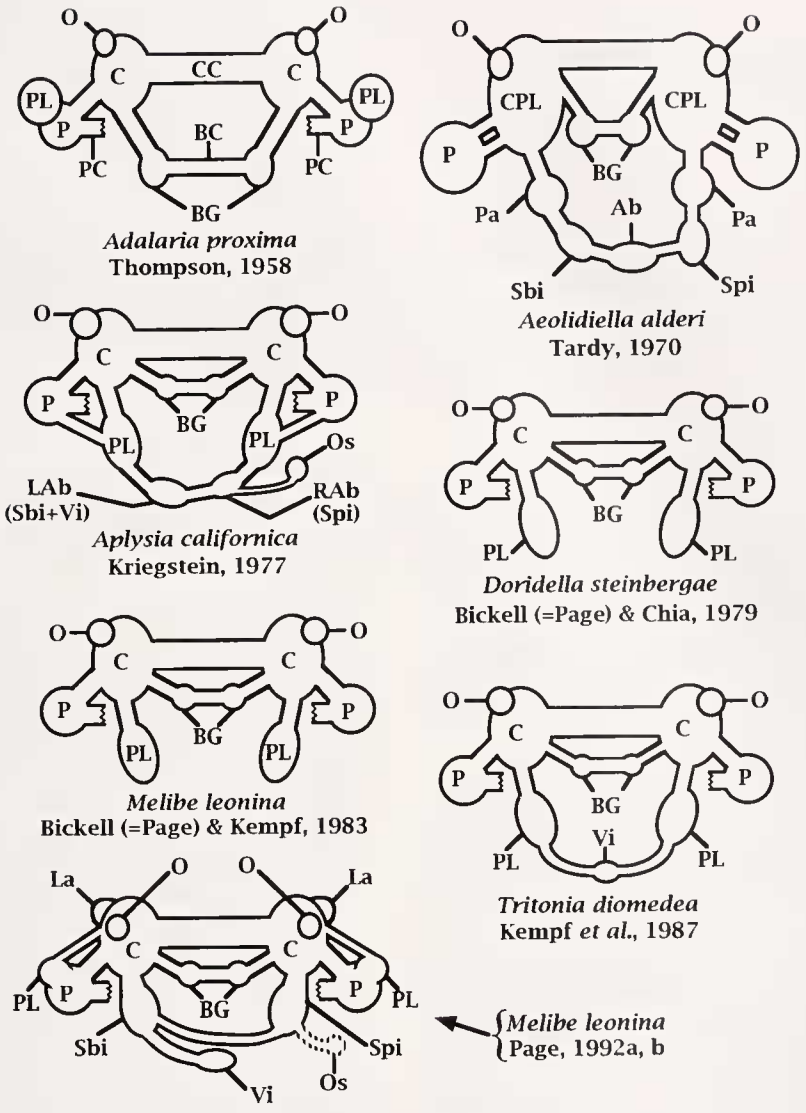

Figure 5. Schematic diagrams illustrating various published descriptions of the central nervous system of different species of competent opisthobranch veliger larvae. Ab = abdominal ganglion; BC = buccal commissure; BG = buccal ganglia; C = cerebral ganglion; CC = cerebral commissure; CPL = cerebropleural ganglion; La = labial ganglion; LAb = left abdominal ganglion; RAb = right abdominal ganglion; O = optic ganglion; Os = osphradial ganglion; P = pedal ganglion; Pa = parietal ganglion; PC = pedal commissure; PL = pleural ganglion; Sbi = subintestinal ganglion; Spi = supraintestinal ganglion; Vi = visceral ganglion.

Acknowledgments

The authors thank Drs. James Bradley and Christine Sundermann for the use of the microtome and photographic equipment, respectively. Barbara Estridge was very helpful, as always, on many matters, but most especially regarding use of the department's new Reichart ultramicrotome. Thanks also to Drs. James Bradley, Raymond Henry, and Curt Peterson for helpful critical comments on the text of this manuscript and to Dr. Yvonne Grimm-Jorgenson for hallway discussions. This research was supported by an Alabama Agricultural Experiment Station grant to S.C.K., AAES Journal No. 15-913124.

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